TECHNIQUES, METHODS and PROCEDURES for
CELL, TISSUE and ORGAN CULTURE

# TCA MANUAL

VOLUME 1

1975

Made in the United States of America
Copyright © 1976 Tissue Culture Association, Inc.
All Rights Reserved

# TISSUE CULTURE ASSOCIATION MANUAL

## Techniques, Methods and Procedures for Cell, Tissue and Organ Culture

*A Publication of the Tissue Culture Association*

EXECUTIVE EDITORS

**Virginia J. Evans, Chairman**
**Vernon P. Perry**
**Monroe M. Vincent**

Biomedical Research Institute
12111 Parklawn Drive
Rockville, Maryland 20852
TELEPHONE (301) 881-8146

1975 BOARD OF ADVISORS

**M. F. Barile**
Bethesda, Maryland

**L. L. Coriell**
Camden, New Jersey

**W. H. J. Douglas**
Lake Placid, New York

**E. M. Earley**
Bethesda, Maryland

**H. B. Fell**
Cambridge, England

**S. Fedoroff**
Saskatoon, Saskatchewan, Canada

**T. D. C. Grace**
Canberra, Australia

**G. M. Healy**
Willowdale, Ontario, Canada

**A. C. Hildebrandt**
Madison, Wisconsin

**H. E. Hopps**
Bethesda, Maryland

**R. N. Hull**
Indianapolis, Indiana

**R. H. Kahn**
Ann Arbor, Michigan

**H. Katsuta**
Tokyo, Japan

**F. H. Kasten**
New Orleans, Louisiana

**A. Leibovitz**
Temple, Texas

**J. Leighton**
Philadelphia, Pennsylvania

**K. Maramorosch**
Yonkers, New York

**D. J. Merchant**
Norfolk, Virginia

**J. F. Morgan**
Saskatoon, Saskatchewan, Canada

**H. J. Morton**
Ottawa, Ontario, Canada

**L. R. Murrell**
Memphis, Tennessee

**M. K. Patterson, Jr.**
Ardmore, Oklahoma

**J. Paul**
Glasgow, Scotland

**K. K. Sanford**
Bethesda, Maryland

**W. I. Schaeffer**
Burlington, Vermont

**J. E. Shannon**
Rockville, Maryland

**R. Sinclair**
Montreal, Quebec, Canada

**J. Sykes**
Los Angeles, California

**J. L. Vaughn**
Beltsville, Maryland

**C. Waymouth**
Bar Harbor, Maine

**K. E. Wolf**
Kearneysville, West Virginia

The TCA Manual, a publication of the Tissue Culture Association, Inc., is devoted to the advancement and dissemination of basic and applied knowledge by publishing techniques, methods and procedures for the cultivation of cells, tissues, organs, or tumors from multicellular animals or plants. The TCA Manual is published quarterly, one volume per year. The subscription price is $35.00 per volume (domestic). Single copies are $10.00 (domestic). Regular members of the Tissue Culture Association may receive the TCA Manual at the reduced rate of $25.00. Correspondence relating to subscriptions, nonreceipt, reprints, defective copies, availability of back issues, advertising, and late or lost proofs should be directed to the Business Office, 12111 Parklawn Drive, Rockville, Maryland 20852 (Area Code 301 881-8146). Application to mail at Second Class Postage Rates pending at Rockville, Maryland 20852.

Made in the United States of America
Copyright©1976 Tissue Culture Association
All Rights Reserved

## NOTICE TO CONTRIBUTORS

1. The Tissue Culture Association Manual is published quarterly by the Tissue Culture Association, Inc. and represents the contributions of investigators in the field of cell, tissue and organ culture. The Manual publishes papers representing techniques, methods and procedures developed in the course of studies with vertebrate, invertebrate and plant culture. Manuscripts should be mailed to:

   Tissue Culture Association Manual
   c/o Biomedical Research Institute
   12111 Parklawn Drive
   Rockville, Maryland 20852

2. Each contribution must have a descriptive title not to exceed fifteen (15) words. The title should be followed by the legend "Submitted by," name(s) of the author(s), and the institutional address. Identify as page 1.

3. Each contribution must have an introduction and be identified as: I INTRODUCTION. The introduction should be brief, yet comprehensive, and include any special instructions, such as CAUTIONS, that the reader should follow. Begin INTRODUCTION on page 2.

4. Each contribution must have a materials section identified as: II MATERIALS. The materials section should list all items to be used in the technique, method or procedure and include a description of the item, a stock or catalogue number and identification of the manufacturer or supplier with a footnote address (the list of footnote addresses should be on a separate page). For example:
   Pipettes, serological 5 ml, No. xxxx Corning[3]

5. Each contribution must have a procedure section identified as: III PROCEDURE. The procedure should be written in the present tense, imperative mood, and should itemize and describe each step to be taken. The stepwise description of this section should be explicit and in such detail that the novice could successfully follow the procedure and duplicate the results of the author(s).

6. Each contribution must have a discussion section identified as: IV DISCUSSION. The discussion should include a description of what can be expected from following the procedure, the role of the procedure in a specific or potential application, and any precautions that should be exercised (for example, in the interpretation of results).

7. Each contribution should have a reference section identified as: V REFERENCES. References must be cited in the text in numerical sequence and listed in Reference section in the order first cited. References should follow the same style as published in the journal IN VITRO. For example:

   1. Danes, B.S., and A.G. Bearn. 1966. Hurler's syndrome: a genetic study in cell culture. J. Exp. Med. 123:1-16.
   2. Price, P.J. 1975. Preparation and use of rat-tail collagen. Procedure 71118. TCA Manual 1:43-44.
   3. Harris, M. 1964. *Cell Culture and Somatic Variation.* Holt, Rinehart and Winston, New York, pp. 233-241.
   4. Trowell, O. A. 1966. Tissue culture in radiobiology. In: E. N. Willmer (Ed.), *Cells and Tissues in Culture. Methods, Biology, and Physiology.* Volume 3. Academic Press, London, pp. 63-149.

8. Each manuscript is to be submitted in an original plus two (2) copies. The first page should include only the title, author(s) and institutional address and should be identified as page 1. Each section, i.e., I INTRODUCTION, II MATERIALS, III PROCEDURE, IV DISCUSSION and V REFERENCES should begin on a separate page. Each manuscript should be typewritten double-spaced, with 1" margins on the right and left and 1-1/2" at top and bottom of each page.

9. The cost of all illustrations, including line drawings, photographs and other art forms, must be borne by the author and should be included only where and when absolutely necessary. The charge for black and white illustrations has been set at $100.00 per page, regardless of size.

10. Subscription rates: Members of the Tissue Culture Association — $25.00 per volume. Non-members — $35.00. Postage and handling charges to non-members — $1.00 Domestic; $2.00 Canada and Mexico; $3.00 elsewhere. No discounts to agents.

11. All inquiries regarding manuscripts, subscriptions, advertising, etc., should be directed to:

    Tissue Culture Association Manual
    c/o Biomedical Research Institute
    12111 Parklawn Drive
    Rockville, Maryland 20852
    Telephone Area Code (301) 881-8146.

Mention of a proprietary product does not necessarily constitute an endorsement of the product by either the author or the Tissue Culture Association. Product sources are given only to indicate what product the investigator used in his particular procedure and to aid the novice in locating a source, or THE source should the novice wish to repeat the procedure exactly.

TISSUE CULTURE ASSOCIATION MANUAL
Techniques, Methods and Procedures for
Cell, Tissue and Organ Culture

Volume 1 Number 1 1975

## TABLE OF CONTENTS


| Procedure Number | Title | Page Number |
|---|---|---|
| 12311 | The laboratory of Alexis Carrel at the Rockefeller Institute. *Submitted by Vernon P. Perry* | 1 |
| 32511 | Hanks' Balanced Salt Solution and pH control. *Submitted by John H. Hanks* | 3 |
| 41117 | A method to grow pieces of superior cervical ganglion *in vitro*. *Submitted by John S. Chen and M. G. Menesini Chen* | 5 |
| 41141 | Preparation of avian cell cultures. *Submitted by J. J. Solomon* | 7 |
| 41523 | Growth of human skin fibroblasts from punch biopsies. *Submitted by Ingeburg E. Goetz* | 13 |
| 41722 | Cultivation of peritoneal mononuclear phagocytes. *Submitted by Susan Tolnai* | 17 |
| 42621 | Primary epithelial outgrowth cultures of keratinocytes from human skin. *Submitted by B. Allen Flaxman* | 21 |
| 44352 | Organ culture of rat colon on fibrin form. *Submitted by Leonard J. Schiff* | 23 |
| 46118 | Preparation of primary chicken embryo liver cells. *Submitted by Frank S. Kohn and Sheila A. Henneman* | 25 |
| 46131 | Technique for mammary epithelial cell isolation and cultivation. *Submitted by Roy C. Foster and Martin K. Feldman* | 27 |
| 61131 | Chromosomal preparations from monolayer cell cultures. *Submitted by Elizabeth M. Earley* | 31 |
| 70111 | A method for viable cell count. *Submitted by Susan Tolnai* | 37 |
| 70151 | Method for the demonstration of phagocytosis by peritoneal mononuclear phagocytes. *Submitted by Susan Tolnai* | 39 |
| 70181 | Vital and cytochemical staining of cell cultures with a fluorescent dye to determine viability. *Submitted by Irwin Katz* | 41 |
| 71118 | Preparation and use of rat-tail collagen. *Submitted by Paul J. Price* | 43 |
| 74544 | Microfluorometric assays for growth of small numbers of cells in culture. *Submitted by Howard L. Hosick* | 45 |
| 78094 | An improved method for single cell cloning and observation. *Submitted by Lucian J. Cuprak* | 49 |
| 81169 | Proposed usage of animal tissue culture terms. *Submitted by S. Fedoroff* | 53 |

TISSUE CULTURE ASSOCIATION MANUAL
Techniques, Methods and Procedures for
Cell, Tissue and Organ Culture
Volume 1 Number 2 1975

## TABLE OF CONTENTS


| Procedure Number | Title | Page Number |
|---|---|---|
| 21684 | The preparation and use of perforated cellophane for tissue culture. *Submitted by Floyd M. Price and Sandra L. Handleman* | 59 |
| 30531 | Preparation and use of dimethylsulfoxide cryoprotective solution. *Submitted by Vernon P. Perry* | 61 |
| 38441 | Preparation of chicken plasma without the use of anticoagulants. *Submitted by Edward L. Schilling and Katherine K. Sanford* | 63 |
| 43121 | Organ culture of mouse limb-buds for study of skeletal development. *Submitted by Margaret B. Aydelotte* | 67 |
| 43133 | Maintenance of embryonic chick duodenum in large scale organ culture. *Submitted by R. A. Corradino* | 71 |
| 43221 | Hamster trachea organ cultures. *Submitted by Michael G. Gabridge* | 75 |
| 46063 | A method of culturing *Drosophila* embryonic cells *in vitro* for nerve and muscle differentiation. *Submitted by J. James Donady and Eric Fyrberg* | 81 |
| 46113 | Isolation of homogeneous cell populations by differential plating. *Submitted by Beppino C. Giovanella, Charles A. Morgan and John S. Stehlin* | 85 |
| 46132 | Preparation of normal and neoplastic rat mammary epithelial cells in monolayer culture. *Submitted by Po-Chuen Chan, Julian Tsuang and Leonard A. Cohen* | 87 |
| 47911 | Human pleural effusions as a source of breast carcinoma cell lines for tissue culture. *Submitted by Relda Cailleau and William J. Reeves, Jr.* | 91 |
| 54099 | A simple method for copy of frames of a 16 mm motion picture. *Submitted by Y. T. Chang* | 95 |
| 54339 | Low-magnification time lapse photomicrography. *Submitted by Y. T. Chang* | 97 |
| 54391 | Long-term time lapse photomicrography. *Submitted by Y. T. Chang* | 99 |
| 60112 | Methylene blue staining of tissue culture monolayers. *Submitted by Sheila A. Henneman and Frank S. Kohn* | 103 |
| 61101 | Toluidine blue staining of cultured animal cells to demonstrate presence of coccidia. *Submitted by W. H. Patton* | 105 |
| 71113 | A procedure for preparing photo-gelled collagen substrates containing Aclar reticles for tissue culture. *Submitted by Edmund B. Masurovsky and Edith R. Peterson* | 107 |
| 75162 | A method for the deionization of bovine serum albumin. *Submitted by Jane E. Barker and Arthur W. Nienhuis* | 111 |
| 75317 | Detection of mycoplasma in cell cultures. *Submitted by Gerard J. McGarrity* | 113 |
| 75423 | Bacteriophage assays of sera. *Submitted by John C. Petricciani* | 117 |
| 76161 | Protected freezing of cell suspensions. *Submitted by Vernon P. Perry, Calvin A. Kroener, and J. Laverne Martin* | 119 |

TISSUE CULTURE ASSOCIATION MANUAL
Techniques, Methods and Procedures for
Cell, Tissue and Organ Culture

Volume 1 Number 3 1975

## TABLE OF CONTENTS


| Procedure Number | Title | Page Number |
|---|---|---|
| 40133 | Organotypic Culture of Early Embryonic Nervous System. *Submitted by Katherine M. Lyser* | 121 |
| 40166 | Establishment of Continuous Organotypic Cultures from Nervous System Tumors. *Submitted by Katherine M. Lyser* | 127 |
| 41177 | Adaptations of the Culture Method of Wolff for Neoplastic Nervous System. *Submitted by Katherine M. Lyser* | 133 |
| 41179 | Envelope Technique for Selective Isolation of Cells from Multilayer Organ Cultures for Metabolic Studies. *Submitted by Abel Lazzarini Robertson, Jr.* | 139 |
| 42641 | Methods for the Development and Analysis of Erythroid Colonies Grown in Plasma Clot Cultures. *Submitted by Jane E. Barker and Arthur W. Nienhuis* | 145 |
| 43811 | Small Glass Dishes for Organ Culture. *Submitted by Stanislawa Stoklosowa* | 149 |
| 46122 | Primary Culture of Leafhopper Embryonic Tissues. *Submitted by Jun Mitsuhashi* | 151 |
| 46127 | Use of Cockerel Testicular Cell Cultures to Study Steroidogenesis and Cell Surface Biology. *Submitted by Harold H. Lee and Lynn Sherman* | 155 |
| 46170 | Primary Epithelial Cell Dome Culture of Mouse Mammary Tumors. *Submitted by L. J. T. Young, R. D. Cardiff and C. M. McGrath* | 161 |
| 53361 | Air-Dry Method for Preparation of Cell Culture Monolayers on Cover Slips for Scanning Electron Microscopy. *Submitted by E. C. Pirtle and J. E. Gallagher* | 165 |
| 75129 | Serum Quality Control. *Submitted by Gerard J. McGarrity* | 167 |
| 75192 | Procedures for Evaluating Growth-Promoting Qualities of Cell Culture Reagents. *Submitted by David Pye* | 171 |
| 78199 | Method for Cloning Plasmacytoma Cells by Attachment to Histone-Coated Plastic Surfaces. *Submitted by Leonard Keay* | 177 |

TISSUE CULTURE ASSOCIATION MANUAL
Techniques, Methods and Procedures for
Cell, Tissue and Organ Culture

Volume 1 Number 4 1975

## TABLE OF CONTENTS


| Procedure Number | Title | Page Number |
|---|---|---|
| 16183 | Control of Microbiological Contamination. *Submitted by Gerard J. McGarrity* | 181 |
| 32512 | Earle's Balanced Salt Solution: Preparation of the Saline. *Submitted by Jay C. Bryant* | 185 |
| 40435 | Rapid and Simple Synchronization of Mammalian Suspension Cell Cultures Involving Minimal Damage to DNA. *Submitted by Thomas W. Sneider and Mary C. King* | 189 |
| 41122 | In Vitro Cultivation of Larval Frog Spinal Cord Explants. *Submitted by Emanuel D. Pollack and Judi Koves* | 193 |
| 41621 | Generation of Cell Lines from Preimplantation Mouse Embryos. *Submitted by Michael I. Sherman* | 199 |
| 43052 | Whole Hamster Trachea Organ Cultures in Laboratory Animal Diagnostic Virology. *Submitted by Leonard J. Schiff* | 203 |
| 46111 | Culture of Follicular Theca Interna Cells. *Submitted by Stanislawa Stoklosowa* | 205 |
| 46172 | Establishment of Cultures from Several Animal Species by Single Trypsin Treatment of Small Tissue Samples. *Submitted by Jean Ferguson Stanley* | 209 |
| 46176 | Reliable and Rapid Procedure for Development of Human Fibroblastic Cell Strains. *Submitted by W.F. Simpson and C.S. Stulberg* | 211 |
| 46513 | Apparatus and Techniques for the Preparation of Cockroach Neuroendocrine Organs for Long-Term Maintenance in Vitro. *Submitted by K. R. Seshan* | 215 |
| 61133 | Chromosome Analysis of Cells in Tissue Culture. *Submitted by Jean Ferguson Stanley* | 219 |
| 61183 | Use of Cultured Cells for in Situ Measurement of Mitochondrial Fragility. *Submitted by Duane G. Wenzel and Daniel Acosta* | 221 |
| 71566 | Recovery of Lactate Dehydrogenase (LDH) from Cell Cultures by Hypotonic Permeation. *Submitted by Byron Th. Aftonomos and John F. Foley* | 225 |
| 75361 | Microscopic Demonstration of Mycoplasma Contamination in Cell Cultures and Cell Culture Media. *Submitted by T.R. Chen* | 229 |
| 94121 | Chemical Analyses of Mammalian Sera Commonly Used as Supplements for Tissue Culture Media. *Submitted by Cecil H. Fox and Katherine K. Sanford* | 233 |
| 99115 | Tissue Culture Mathematics. Cell and Media Concentrations and Volumes. *Submitted by Aaron E. Freeman* | 239 |
| 08985 | Perfusion and Mass Culture Systems. *Submitted by M.K. Patterson, Jr.* | 243 |
| | Author Index | 251 |
| | Subject Index | 252 |